# Biological Interactions: Effects on and the Use of Soil Invertebrates in Relation to Soil Contamination and In Situ Soil Reclamation

**Mischa B.M. Indeherberg, Alain J.P. De Vocht and Cornelis A.M. Van Gestel**

In recent years, soil invertebrates attracted the attention of researchers in taxonomy, ecology, agriculture, toxicology, biochemistry and other disciplines. It has been realised, not only from a taxonomic point of view, that soil invertebrate diversity is enormous. From an ethical point of view but also because of our own human interest there is a need to study and preserve soil invertebrates to protect biodiversity. Although their functioning in soils is largely unexplained, it has been shown that many soil invertebrates have a large impact on soil structure and soil fertility and mediate in decomposition and mineralization processes.[1] It is clear that soil invertebrates should be taken into account to improve soil management for sustainable agriculture or to prevent erosion. On the other hand, ecological processes in which soil invertebrates take part are vulnerable and can be affected by many kinds of contaminants.[2,3] The upper soil horizon and litter layer form the major sink for heavy metals originating from human activities.[4,5] Moreover, as many soil invertebrates are surface dwellers, they will be exposed to much higher doses of contaminants than would be expected on the basis of a homogeneous distribution of contaminating substances.[6] In the last decades, much of information has been gained toward estimating to what extent soil invertebrates are affected by heavy metal pollution and whether defense mechanisms exist that allow for survival under these hazardous circumstances. Many questions arise in view of the evaluation and application of soil improvement techniques such as in situ inactivation and phytorestoration, . Are soil invertebrate populations surviving at metal contaminated areas able to (re-)adapt to a habitat with reduced metal availability? How should we use soil invertebrates to evaluate the efficiency and durability of soil improvement techniques? In this chapter we will try to give some elements of answers to these questions. We will also give arguments to stimulate future research by suggesting some working methods to answer these questions.

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We will first demonstrate the diversity and the importance of the soil invertebrate community. In section 7.2, we address whether adverse effects on soil invertebrates due to heavy metals indeed are adverse enough to motivate the reclamation of these areas. In section 7.3, the exposure route within this animal group will be discussed in regards to what extent soil invertebrates can be expected to act as a uniform target group for heavy metals. Whether the soil meso- and macrofauna is able to counteract adverse effects by acclimation and adaptation will be dealt with in section 7.4. Finally, we will propose some methods to use soil invertebrates as an instrument for the evaluation of soil improvement activities, both by means of toxicity testing and in situ biological monitoring.

## 7.1. Diversity and Importance of Soil Invertebrates

### *7.1.1. Soil Invertebrate Diversity*

Terrestrial soils form an important environment where biogenetic processes, such as transformation, decomposition and mineralization of organic matter, take place. Therefore, all ecological groups, most phyla and a wide variety of species can be found living permanently or temporarily in soils.

Soil invertebrates can be classified according to their taxonomy, size, life-history, trophic level, physiology or their soil microhabitat. The most common taxa inhabiting soils are bacteria, protozoa, nematodes, Lumbricidae and Enchytraeidae (*Oligochaeta*), springtails (*Collembola*), mites (*Acarina*), millipedes and centipedes (*Myriapoda*), woodlice (*Isopoda*), snails (*Gastropoda*) and spiders (*Aranea*) (Fig. 7.1). Phylogenetic relationships can be important for ecotoxicological approaches because a classification based on morphological features is likely to group species similar in toxicology reaction pattern as well.

Soil animals can be classified by size by dividing them into micro-, meso- (or meio-) and macrofauna. Microfauna comprises species such as protozoa and some nematodes (<200 μm). Mesofauna averages between 200 μm and 10 mm and comprises taxa such as Nematoda, Collembola, Acarina and Enchytraeidae. Macrofauna is larger than 10 mm and comprises larger Oligochaeta (Lumbricidae), Gastropoda and many arthropod taxa, such as Chelicerata, Isopoda, Insecta and Myriapoda. This classification corresponds more or less to the one proposed by Pokarzhevskii[7] distinguishing three 'ecosystems' within the soil community. The size of soil organisms is related to the microhabitat of these species in the soil and will affect their exposure risk (section 7.3.3). In this chapter we will mainly concentrate on the meso- and macrofauna (Fig. 7.1).

Soil organisms can also be classified in view of their life strategy. r- and K selected species can be distinguished. r-Selected species are characterized by a high productivity, large intrinsic growth rate, a short life-span and a high mortality of the juveniles. K-selected species exhibit a low productivity, small intrinsic growth rate, a long life-span and a small overall mortality of the juveniles.[8] Oribatid mites for instance, although generally very small (1 mm or less), can be regarded as K-selected organisms.[9] Within springtails and earthworms, different life-history strategies may be related to their vertical distribution within the soil top layer. A subdivision into epigeon, hemiedaphon and euedaphon species, referring respectively to species living on the soil, in the upper few centimeters of the soil and deeper in the soil, has been proposed for springtails and earthworms.[10,11] This division into different life-forms seems to match with different life-history strategies. The surface-active species have a higher metabolism and higher fertility compared with true soil inhabitants and should therefore be considered as rather r-selected species. The recovery capacity of a species after soil pollution may depend largely on its life-history pattern.



Soil invertebrates also comprise a diversity of trophic groups. Detrivorous, bacterivorous, fungivorous, herbivorous, carnivorous and omnivorous taxa inhabit the soil ecosystem. Whereas spiders (Aranea) are all carnivores, other taxa are known to depend on a variety of food items. Oribatid mites, for instance, have members which feed on fungi, algae, lichens, bacteria, protozoa and even nematodes.[9] The food source, together with the microhabitat and some morphological characteristics, should be considered when evaluating the soil metal fraction that has to be considered for uptake (see section 7.3.2).

Finally, soil invertebrates could be separated based on their physiological and biochemical features. This will be discussed in section 7.3.3.

### *7.1.2. Impact of Soil Invertebrates on the Soil Ecosystem*

Litter dwelling millipedes, woodlice and earthworms influence the top layer of soils significantly. Earthworms ingest up to 25% of the humic layer of the soil per year.[12] They affect the nutrient cycling in soils and the carbon flux in and out soils. Their burrowing and feeding activities influence metal speciation and availability and microbial composition of the soil. Earthworms can play an important role in the regulation of bacterial composition and densities and thereby also microbial-mediated processes.[13] It has been demonstrated that earthworms negatively affect the growth of plants by supporting the introduction of pathogenic Pseudomonads to the root area. They can also fortify the effects of biocontrol agents of root disease.[14,15]

Other taxa are also known to affect the bacterial composition of the soil. The total number of bacteria in excrements of soil invertebrates is often higher than in the food. For example, many bacteria decrease in number in the midgut but those that survive multiply rapidly in the hindgut. Important differences in survival of gut passage through earthworms and woodlice are found between different bacteria and bacterial strains.[16-18] Saprotrophic and microbivorous soil invertebrates stimulate the microflora in their functioning directly and indirectly. Verhoef and Brussaard[1] estimated that soil fauna accounted for about 30% of the nitrogen mobilization. A decreased litter decomposition following soil contamination is indirect proof of the importance of soil biota.

## *7.2. Field Observations of the Impact of Metal Contamination on Soil Invertebrates*

Before considering the application of in situ inactivation and phytorestoration techniques we should ask whether soil invertebrate communities are really negatively influenced by heavy metal contamination. And if so, is it necessary to bother about it? In section 7.1.2 the importance of soil invertebrates in the soil ecosystem and their economic importance in soil fertility processes was mentioned. In this section, an overview of the knowledge of the impact of heavy metal contamination on soil invertebrates will be given. As suggested by Hopkin,[19] effects on four different levels can be distinguished.

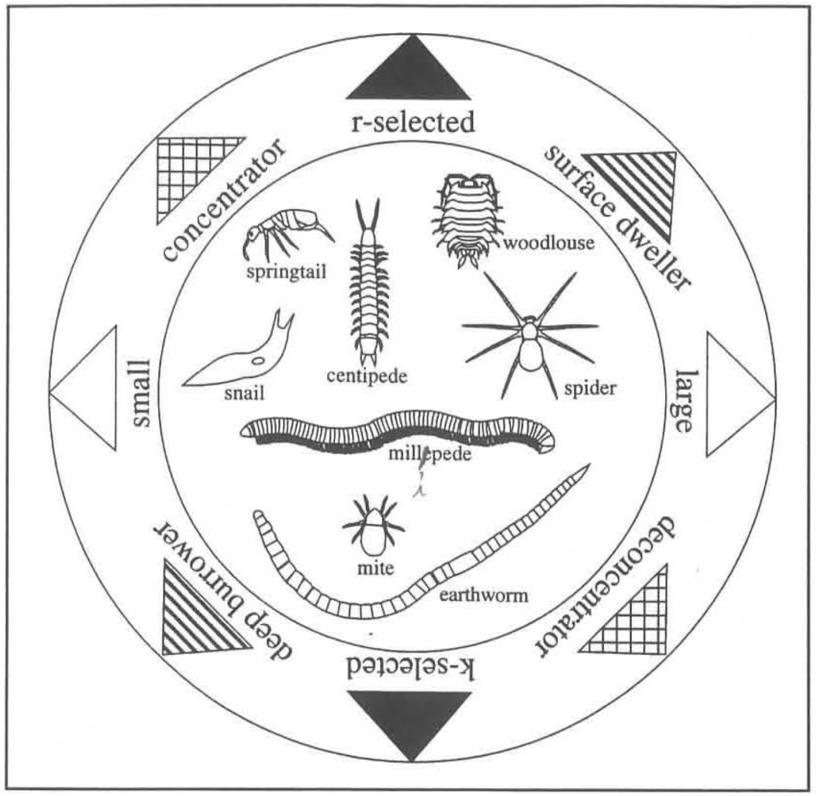


Fig. 7.1. Abstraction of the diversity encountered within soil (meso- and macro-) invertebrates. A dichotomous division of soil invertebrates concerning a certain feature, and the number of taxa presented are a strong simplification of reality.

### *7.2.1. Community Effects*

Does heavy metal contamination affect the presence or absence of certain species? Has a more subtle effect on density of species been shown? For springtails (Collembola) and spiders (Aranea), there is some evidence that they maintain their density in metal-contaminated sites.[20,21] For springtails, the repeated moulting process that leads to a high excretion efficiency of metals due to the renewal of the midgut epithelium is probably the most likely explanation.[22,23] The species composition of springtails, however, was shown to change gradually in the direction of a source of contamination.[20] Differences in the food specificity, resistance level, reproduction biology and dispersal abilities within Collembola species are assumed to be the main reasons for their site-specificity.[24,25] A real restriction of particular species to heavy metal-contaminated soils, as observed for certain plant species,[26] cannot be concluded. In general, this can probably be explained by the existence of tolerance mechanisms that are alterations of degree rather than kind.[27]



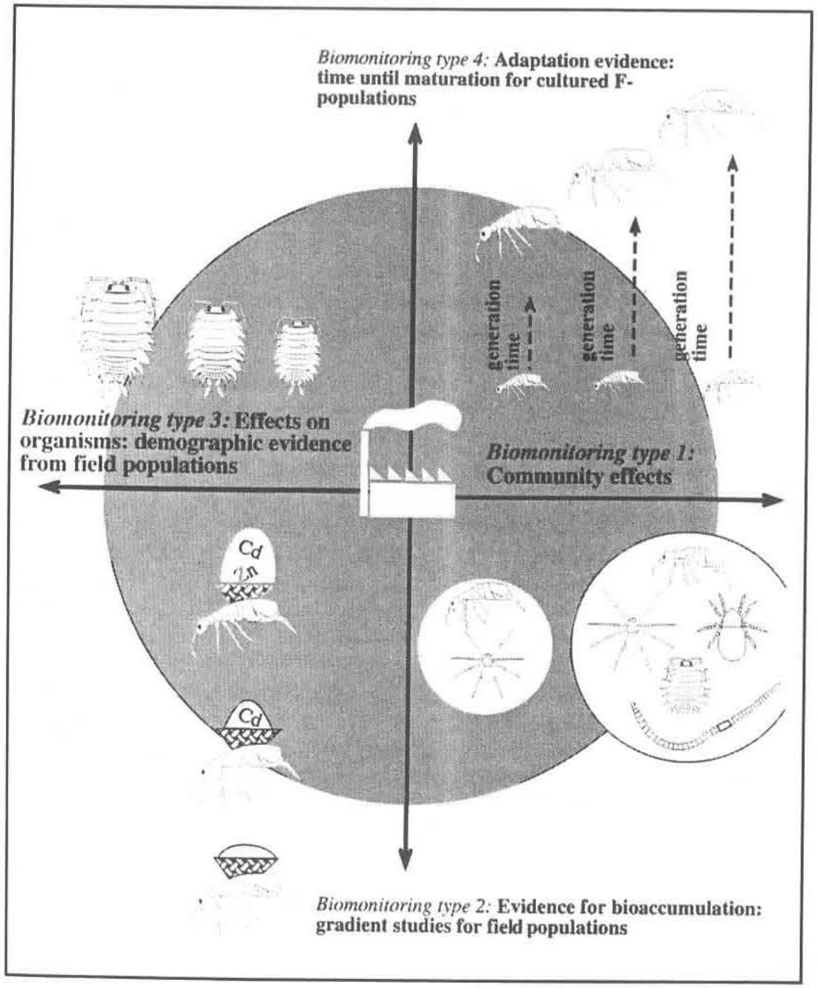


Fig. 7.2. The different ways of in situ biological monitoring demonstrate significant adverse effects on soil invertebrates in the direction of the heavy metal pollution source symbolized as a picture of a factory.

In most studies, it has been shown that population densities and/or species number decrease significantly in the direction of a metal-contaminated location (Fig. 7.2). This inverse correlation with the degree of metal contamination has been demonstrated for different kinds of metal contaminated areas such as: natural (Pb)-contaminated sites,[28] Pb and brass smelter sites,[29,30] and Pb-contaminated roadsides.[31] Adverse community effects are known to occur for most taxa: earthworms[21,29,30] terrestrial isopods,[21] springtails,[20,28] arachnids,[21,32] and oribatid mites.[28,33]

### 7.2.2. Concentrations of Metals in Soil Invertebrates at Metal-Contaminated Sites

Elevated internal heavy metal concentrations at metal-contaminated sites have been shown to occur for several taxa: springtails,[5,34,35] earthworms,[29] spiders,[36] woodlice,[37,38] pseudoscorpions, mites, harvestmen and carabids[34] (Fig. 7.2).

However, this rule cannot be applied for every metal species. Donker[37] reported enlarged metal body concentrations for the isopod *Porcellio scaber* at contaminated sites for all the metals investigated (Cu, Pb, Zn). At a smelter site, Janssen and Hogerhorst[34] did detect a significant increase of internal Cd and Zn concentrations for springtails, pseudoscorpions, mites, harvestmen and carabids whereas internal Cu and Pb concentrations remained at the reference level. Therefore, if an increased metal concentration for soil invertebrates is restricted to only a few metal species, these are not necessarily non-essential elements. Although a significant increase in the internal metal concentrations at contaminated sites may be regarded as a general rule, the extent to which this metal concentration increases is taxon dependent. Differences in Cd and Zn concentrations for reference sites and contaminated areas turned out to be larger for Isopoda than for Collembola sampled at the same sites.[35,37] Additionally, increased internal metal concentrations as observed for contaminated sites do not necessarily correspond with negative effects on the individual or population level. In fact, such measurements first inform us as to whether the contaminated area actually leads to an increased external availability (section 7.3.2) of heavy metals when compared with carefully chosen reference sites. It is presumed that internal threshold concentrations exist, which determine the adverse effect that can be expected on the individual and population level.[39] The determination of such critical body concentrations for soil invertebrates is a major challenge and allows for determination of risks towards biota (see section 7.5.2).

### 7.2.3. Effects of Metals at the Individual and Population Level as Observed in the Field

Negative effects of heavy metals in a field situation have been shown for the isopod *Porcellio scaber*.[37] Here, energy reserves were compared for field animals originating from a reference and two contaminated sites. Reduction of energy reserves was observed for the largest individuals of the most exposed population. A demographic study on the same species revealed a reduced size of the individuals (Fig. 7.2) at contaminated sites that resulted in a lower percentage of gravid females and a delayed peak of reproduction. Compared with the reference site, the reproduction expressed as the weight of the brood divided by the weight of the mother, increased.[40] In comparison with a reference site, growth of adults and juveniles, as well as cocoon production for the earthworm *Dendrobaena octaedra*, was seriously reduced at a brass mill. It was concluded that the low population density of this species was due to immigration of individuals from adjacent areas or heterogeneity of metal distribution, allowing for survival of individuals at limited sites within the area.[30]

### 7.2.4. Adaptation of Species as an Indicator of Long-Term Pollution Effects

When a population living at a contaminated site shows genetically determined differences in its biochemistry, physiology or life-history, allowing for a better chance to survive in this hazardous environment when compared with a reference



population, it can be considered as adapted to heavy metals. When evidence is found for the existence of adaptation mechanisms, this can be regarded as a result of natural selection due to the heavy metal stress factor. Adaptation mechanisms detected for soil invertebrate group will be discussed in section 7.4. Adaptation evidence has been found for the isopod *Porcellio scaber* [41] and the springtail species *Orchesella cincta*,[22,35,42] *Onychiurus armatus* and *Isotoma notabilis*.[43]

## 7.3. Metal Pathways in Soil Invertebrates

### 7.3.1. Introduction

In recent years more attention has been given to the relationship between availability and toxicity of metals to soil organisms. In this context the theoretical concept 'bioavailability' is defined as the extent to which contaminants from the environment can enter the tissue of the soil organism. Bioavailability is influenced by characteristics of the environment, the metal and the biological species considered. In this chapter, within this theoretical concept—bioavailability—the external and the internal available fraction, will be distinguished. The soil metal fraction that has to be considered for uptake into the organism as a whole is referred to as the external available fraction. This fraction depends on the environment, the metal and the biological species itself. In parallel, the internal available fraction is the amount of metals available to exert an effect inside. Measurements such as external metal concentrations using soil extraction techniques and internal body concentrations integrate aspects of bioavailability in different ways. After entering the body tissue, physiological processes affect the distribution and the prolonged stay of the metal in an individual organism.

Internal metal concentrations are the result of both internal and external availability and the physiological (defense) mechanisms which take place after the metals enter the body tissue. Using internal concentrations as a measure for the bioavailability of a contaminant is only valid when some physiological features of the organism are met (section 7.3.4).

The external available fraction of metals for a specific soil invertebrate can be estimated using soil extraction techniques. The microhabitat, size, trophic level and food specificity are species-related and result in differences in the external available fraction for different soil invertebrates living at the same site in the same soil.

### 7.3.2. Metal-, Environmental- and Species-Determined Differences of the External Available Fraction

Heavy metals can be present in the three phases of the soil (solid, aqueous, gaseous) and in many forms, such as unsolved mineral form, unsolved adsorbed to clay minerals and humic compounds, unsolved complexes with different organic molecules or iron, manganese or aluminium oxides or solved as free cation and anion-cation complexes.[44,45] Many interacting physico-chemical soil parameters affect metal availability to soil organisms, therefore the external available fraction in the soil is very complex and hard to quantify.[46,47] Different soil properties determine the availability and toxicity to soil invertebrates for each metal. The importance of the impact of soil parameters on the metal toxicity depends on the metal and the biological species.[48,49] Of all soil variables, the cation exchange capacity and pH should be the most important descriptors for metal toxicity to soil

invertebrates. In general, metals in soils are available to soil invertebrates in solved forms, especially as free cations. The relation or equilibrium between total soil concentration and pore water concentration is determined by physico-chemical and biochemical soil characteristics. The behavior of metals in respect to soil characteristics is metal-specific, resulting in a metal-specific solubility in the pore water.[44,50,51] If metals are mainly available through the aqueous phase of soils, especially adsorption and desorption will affect the availability of free metal ions. Pore water or water soluble concentrations have proved to be more accurate than total concentrations in soils and explain the toxicity of Zn for springtails and earthworms.[52,53] The pore water hypothesis[48] is confirmed from these results. This hypothesis implies that the solved concentration of a metal determines the toxic effects and not the total amount of metal in the soil.

Time and the effects of aging, weathering and land use are important factors in determining differences in soil metal concentrations and bioavailability to different populations of soil invertebrates.[52] Changing soil characteristics can also influence the exposure route of soil invertebrates. An increased pH was found to result in an increased adsorption of Cu to yeast cells in comparison to the soil particles.[54] As a result, the relative importance of oral uptake is supposed to increase in comparison with dermal metal uptake.

Ingestion of particles followed by cellular assimilation and dermal uptake are the most important ways for metals to enter the body tissue. Inhalation of vapors in soils is a possible exposure route for species which possess book lungs or tracheae but this is not mentioned in the literature. Microhabitat, body size, trophic level and food specificity are species-related factors. These factors can disturb the correlation between pore water concentrations and soil metal concentrations (using soil extraction techniques) and toxic effects of heavy metals. The extent to which a soil invertebrate taxon is related to the pore water by means of its microhabitat results in differences in the external available fraction. Body size, as a second species-specific feature, has been shown to correlate positively with the internal Pb concentration. Cd and Zn concentrations were not related to body size.[55] The feeding habit also determines to a great extent the external available fraction. Metals bound to soil components are much more likely to enter the body of a detritus feeder than a predator. Some species are able to discriminate between food with a high or low metal content.[24,25,56] This behavior obviously affects the metal fraction that will be ingested. Differences in activity will affect the external available fraction as well.

The physico-chemical properties of the gut contents determine the uptake of metals into the body tissue. Little is known about the impact of gut microflora and physico-chemical conditions of the digestive system on the assimilation of metals. Calcium was found to be the most important biochemical factor that influences the accumulation of Pb in earthworms.[57] Mechanisms such as competitive absorption of calcium and the inhibition of transepithelial transport of Pb are thought to be important factors in reducing Pb assimilation. Processes that influence the bioavailability of metals in the digestive system of deposit-feeding invertebrates are believed to be identical to processes controlling sorption and desorption processes in soils.[58]



### 7.3.3. Concentration and Deconcentration of Metals in Soil Invertebrates

Several studies indicate the existence of large differences in body concentrations between related species gathered from the same contaminated area.[29,55] This is probably due to another factor affecting internal concentrations in soil invertebrates: toxicokinetic characteristics. The uptake ($k_1$) and elimination ($k_2$) rate constants as well as the internal threshold concentration for toxic effects of a species, can be regarded as more or less independent of environmental characteristics. $k_1$ And $k_2$ values have been estimated within soil invertebrate species using different experimental strategies.[59,60] The uptake rate constants for two Collembola and one oribatid mite species fed with metal-contaminated algae under the same laboratory conditions did not differ much. A wider range was found in the metal elimination rates within these species.[59] The metal elimination rate of a species is an important parameter determining its regulation strategy. This strategy can be located somewhere between a pure storage on the one hand and a strict elimination from the body tissue on the other hand. Both mechanisms result in different internal metal concentrations. Based on the body to soil concentration factor for heavy metals, Dallinger distinguished macroconcentrators, microconcentrators and deconcentrators within soil invertebrate species.[61] Distinct differences in concentration factors are found for related species in some taxa, such as gastropods or mites. It should also be mentioned that within a species, different elimination strategies can appear for different heavy metals. As will be pointed out in section 7.5., it is important to know where a taxon or species should be classified according to its uptake and elimination strategy.[62]

### 7.3.4. Measuring Bioavailability in Practice

In practice, two different approaches are mainly used to estimate bioavailable concentrations: (a) water soluble, calcium chloride or ammonium acetate exchangeable or metal concentration in the pore water are used as a relative measure of external available concentration in soils;[52] (b) internal metal concentrations can also be used as a relative measure of bioavailability. The measurements of the external concentrations only depend on the environmental and metal aspects. Species-related factors are not integrated in these estimations of bioavailability. The extracted fraction depends on the method used. Although not described exactly, it is assumed that differences in the extracted amount depend on the speciation forms of a metal that can be extracted by a certain method. This implies that the best extraction method for a given situation depends on the metal and biological species studied. It has been demonstrated that good correlations exist between the total metal concentrations of the soil and internal metal concentrations of earthworms.[63] Feeding habit and motility of earthworms allow them to come into contact with the soil as a whole. In contrast, because of the microhabitat occupied by the springtail *Folsomia candida*, more refined techniques should be used.[52] Determination of different soil variables, such as pH, organic matter, clay content and CEC are important when different techniques are used to estimate external availability from soils. In order to improve comparison of data, the standardization of these techniques is important. All aspects related to external availability measurements must be taken into account, especially when bioassays, in which field soils are used, are adopted for the evaluation of soil restoration. Soil parameters not only affect uptake and toxicity of heavy metals but also have a direct effect on the endpoint chosen in toxicity studies such as growth or reproduction.[64]

Internal concentrations in the animal as a whole or of specific tissues or organs can be used to integrate both species-related and soil-related aspects of bioavailability. In practice, the internal concentrations are measured as total concentrations in whole organisms or in specific organs after digestion. It must be mentioned that only a fraction of the total metal body burden will interfere with biological functioning and that only this fraction interfering with the biological processes will cause adverse effects. Adverse effects of heavy metal contamination on soil invertebrates are assumed to occur when species-specific internal threshold concentrations have been exceeded.[39] Recently, it has been indicated that this assumption does not hold for strongly regulated essential elements such as Zn. In *Folsomia candida*, no internal threshold concentration could be determined above which vital functions were affected.[52]

The most appropriate method to describe bioavailability will depend on the biological and metal species considered and on the aim of the experiment. It should be realized that soil treatment with additives essentially affects soil invertebrate body concentrations by affecting external available metal fractions. Test organisms, in the field or in bioassays, can often be used as monitors for bioavailability. For essential metals and in cases of significant metal elimination, external concentrations in soils often are a better indicator for bioavailability and toxicity than internal concentrations.[39] Internal concentrations approximate the extent to which heavy metals are available to the body tissue when elimination of the metal is limited. The measurement of internal body concentrations can be very useful when internal concentrations can be related to adverse effects.[39] This allows for a direct estimation of the severity of contamination (section 7.5.1).

## 7.4. Mechanisms Allowing Soil Invertebrates to Cope with Metal Contamination

### 7.4.1. Cellular Mechanisms: Introduction

Both detoxification by excretion and detoxification by storage of metals can be regarded as regulation mechanisms. Both mechanisms to maintain homeostasis result in different internal metal concentrations. From differences in concentration factors it is clear that soil invertebrates possess cellular mechanisms for metal assimilation, elimination, immobilization or storage in order to maintain homeostasis.[61] These mechanisms allow acclimation or adaptation to environmental heavy metal stress.

At the biochemical level, the major intoxicating effects of heavy metals are: (a) binding to proteins (amino-groups, sulphohydrylgroups), (b) substitution of other metals in metallo-enzymes and inactivation of enzymes, (c) binding to metal carriers of ion pumps, (d) catalysis of free radical generation and (e) binding to nucleic acids with mutagenic effects on DNA or disturbance of protein synthesis at the RNA level.[65]

Heavy metals have been shown to induce the synthesis of stress proteins in soil invertebrates. Two major families of stress proteins have been identified in soil invertebrates: 'heat shock' proteins (Hsp) and stressor specific stress proteins, including metallothioneins and other metal binding proteins.[66] The latter group is considered to function in the detoxification of metals.



### 7.4.2. Maintenance of Homeostasis

To avoid the toxic effects of (an excess of) heavy metals within the body, different strategies can be distinguished on the biochemical and physiological level. Under normal conditions and low stress levels, a constitutive gene expresses an Hsp which functions as molecular "chaperone".[67] Under stress conditions, an inducible gene expresses stress proteins that stabilize or repair protein structure. In this respect Hsp72 is important and has only been found to be inducible by stress factors, especially heavy metals. Hsp72 has been found to resolve denaturated pre-ribosomal complexes and helps to restore nucleolar function.

Proteins belonging to the Hsp70 family have been proposed to be used in monitoring environmental toxicants in soil invertebrates.[68] They have recently been characterized and induced in millipedes,[69] nematodes,[70] slugs and earthworms.[71]

### 7.4.3. Storage-Detoxification and Elimination

Stressor-specific proteins participate in the biochemical processing of chemicals or their metabolites. Metallothioneins or related metal-binding proteins have been studied in soil invertebrates. Metallothioneins possess a high content of sulfur (cysteine) and can bind to Cd, Zn and Cu with a cluster of thiolate bonds.[72] Synthesis is induced by exposure to heavy metals and mainly regulated at the level of transcription initiation.[73] The metallothionein molecular detoxification mechanism acts by inactivating toxic metals or metal concentrations in the cytoplasm of soil invertebrates. However, metallothioneins are not always found in soil invertebrates and often other metal binding proteins or glycoproteins are encountered.[74-76] Findings of independent binding of Cd and Zn to different proteins, stresses the theory that metallothioneins function mainly as detoxification mechanisms in soil invertebrates. Detoxification of heavy metals by metallothioneins has only been documented in earthworms[77,78] and snails.[79,80]

Findings in soil invertebrates mainly support the theory that metallothioneins (if present) participate in detoxification mechanisms. Janssen and Dallinger[80] found that cadmium (Cd) and Cu were bound to cytosolic components. The synthesis of different proteins in the midgut gland of a terrestrial snail was found when exposed to different levels of Cd in food. The newly formed metallothioneins prevent metals from binding to other essential metalloproteins. The high affinity of metallothioneins for Cd and the long half-life of the Cd-containing protein make it suitable for detoxification.[73]

The sparse information available on molecular strategies of metal detoxification hints at the existence of two different strategies. Elimination of metals can be established either by storage-detoxification or excretion. The mechanisms originally described for the isopod *Porcellio scaber* and the springtail species *Orchesella cincta* are not always clearly separated. Lauverjat et al[81] found that metal-loaded metallothioneins were sequestered in lysosomes in *Drosophila*. Similar lysosomes and mechanisms have been described and proposed for *Porcellio scaber*.[82,83] In *Helix pomatia*, however, high levels of metallothioneins were retained in the cytosol over long periods of time.[61]

As pointed out before, accumulation of Cd and Zn in intracellular vesicles have been described for various taxa of soil invertebrates. Brown[84] described three types of metal-containing granules in invertebrate tissues—each one associated with either ferritin, Cu or Ca. The presence of these three types of granules or vesicles have been reported for earthworms,[85,86] isopods,[82,87,88] springtails,[89-91] snails,[92] and

spiders.[93] In macrofauna accumulation has been shown to be concentrated mainly in the digestive tissues, such as the intestinal epithelium or digestive glands, leaving other tissues unaffected.[76,84] Strong evidence exists that all three types of metal-containing vesicles found form part of the lysosomal system.[61] Type B vesicles possess an irregular, amorphic structure and have been shown to be involved in the sequestration of Cd, Cu, Pb and Zn bound to sulfur-rich proteins such as metallothioneins.[81,83,88] Type C vesicles contain crystalline inclusions or flocculant contents and have been reported from hepatopancreas B-cells of isopods and digesting hepatopancreatic cells in spiders.[88] These vesicles are probably also lysosomal and contain degradation products of ferritin.[61] They have been shown to contain Zn and Pb as well.[88] Type A vesicles have a characteristic arrangement of concentric layers and contain both high amounts of Ca and P. Apart from their discovery in concentrators, such as spiders, they have been detected within the gut pellet of springtails as well.[88,89] Zn and Pb have been detected in these vesicles.[88] According to Dallinger,[61] all three types of vesicles form part of the lysosomal system. This implies that the universal lysosomal system contributes to heavy metal acclimation or adaptation within soil invertebrates. Such indications are present for metallothionein functioning as well.

Measurements of excretion efficiency (see section 7.3) in populations of the springtail *Orchesella cincta* can be brought in correlation with the cellular mechanisms. A greater ability of the midgut epithelium to sequester metals in lysosomes and excrete these metals in the gut pellet, results in a higher excretion efficiency.[22] By excreting the gut pellet, springtails are able to excrete a high amount of heavy metals, such as Cd, from the body. The heritability of the cadmium excretion efficiency has been demonstrated for a reference population. A significant positive correlation was shown between the excretion efficiency of the male parent and the excretion efficiency of its offspring.[93] The Cd excretion efficiency has been shown to be elevated for several populations with a metal exposure history. However, the genetic variation within the adapted population decreased in comparison with a reference population. In a sense, the specialization of such populations makes them more vulnerable when the habitat is disturbed one way or another. This may be of importance when attempts are made to restore heavy metal-contaminated sites using techniques such as in situ inactivation and phytoextraction (both resulting in a decreased external available metal fraction).

### 7.4.2. Life-History Adaptations

As described in section 7.1.1, differences concerning the life-history pattern exist between and within soil invertebrate taxa. It has been put forward that disturbances in the habitat could result in evolutionary changes of life-history parameters within a species.[8,94] This hypothesis was tested for several terrestrial invertebrates by comparing populations of a certain species with a different heavy metal exposure history. Research was carried out for the springtail species *Orchesella cincta*, *Onychiurus armatus* and *Isotoma notabilis* and the isopod *Porcellio scaber*. Evidence came from two experimental designs. First, reproductive features were compared between F-populations descending from differently exposed field populations cultured under normal conditions. F1-populations originating from long-term heavy metal-contaminated locations showed early reproduction (Fig. 7.2) and an increased investment in reproduction compared to reference populations.[41-43] The higher reproductive allocation, however, was accomplished by different mechanisms. *Onychiurus armatus* demonstrates a higher number of clutches whereas *Orchesella cincta* and *Isotoma notabilis* show a higher number of juveniles. For the isopod *Porcellio scaber* the reproductive allocation, defined as the mean weight of the offspring divided by the mean weight of the mother, was enlarged for populations originating from contaminated areas.



Second, reproductive characteristics have been quantified with increasing heavy metal exposure for laboratory cultured F-populations originating from differently exposed field populations. When exposed to high metal concentrations, differences in reproductive investment between reference populations and populations originating from long-term exposed sites were even more pronounced.[42,43]

Apart from evidence of reproduction biology, sufficient proof has been given for a genetically determined increased growth rate for long-term exposed populations.[35,41,43]

In this context, we refer to some studies indicating a reduced survival potential of (adapted) populations cultured on soils with a low metal availability.[42,52] Whether a lower performance of populations inhabiting metal-contaminated soils after soil treatment has to be regarded as a general rule is unlikely. Considering their reproductive capacity, adapted populations of the springtails *Onychiurus armatus* and *Isotoma notabilis* are not inferior to reference populations when cultured in an uncontaminated environment.[43]

## 7.5. The Use of Soil Invertebrates to Evaluate In Situ Inactivation and Phytorestoration of Metal Contaminated Soils

### 7.5.1. In Situ Biological Monitoring (Related) Techniques

As discussed in section 7.2, soil invertebrates have been used to evaluate metal-contaminated sites in situ. The strategies applied to investigate possible adverse effects can be classified as different in situ biological monitoring techniques. Hopkin[19] describes them as techniques to assess effects of contaminants on organisms that are collected directly from contaminated sites in the field, or transplanted to or from a contaminated site. In situ biological monitoring is a way to perform pollution assessment but it cannot be carried out routinely as the experimental design is case-dependent and thus time-consuming. Relatively quick and cheap application procedures are not yet available. Nevertheless, in situ biological monitoring gives a rather realistic picture of the impact of metal-polluted sites on an individual, species and ecosystem level.

To estimate whether a certain species diversity, internal metal concentration or energy reserve level at a metal-exposed site should be considered as normal, a comparison with a reference site is helpful. Selecting a proper reference site is often the most difficult problem encountered during biomonitoring studies, as several conditions should be fulfilled. A reference site should demonstrate a low toxicity of metals and other contaminants that cannot be derived easily from soil contaminant measurements (7.3). Furthermore, it should be noted that the performance of populations on reference sites and contaminated sites is, apart from contaminants, determined by a large set of biotic and abiotic characteristics of the soil. Habitat characteristics should therefore be as similar as possible for both sites. In practice, finding such combinations of contaminated and reference sites is nearly impossible. To overcome this problem, many biological monitoring studies are

performed using several sites on a contamination gradient.[20,28,29,52] This way, effects not corresponding to the gradient can be attributed to variables not related to contamination level.

In the context of the evaluation of soil improvement techniques, the problem of finding a proper reference site is less urgent. In situ inactivation of metals, for instance, can be applied to a part of a more or less homogeneous heavy metal contaminated area. The untreated (contaminated) area could serve as an excellent reference area.

Guidelines for the use of biological monitoring applications using soil invertebrates, with an aim toward validating soil improvement techniques, are given below. In general, in situ biomonitoring after in situ inactivation or phytorestoration can be performed by the comparison of treated and untreated areas during one occasion or by following the treated soil over a prolonged period of time.

7.5.1.1. Density and Species Composition

Examining the abundance, species number and species diversity at several locations for each taxonomic group is one way to detect community effects. However, an effective and less laborious solution has been proposed as selecting only one taxonomic group. As suggested by Pearson,[95] faunal groups suitable for community monitoring studies should meet several conditions: 1) taxonomy should be well known and stable, 2) natural history should be well known, 3) they must be readily surveyable and easy to manipulate and identify, 4) higher taxa must have a broad geographical distribution over a wide range of habitat types, 5) lower taxa should be specialized and sensitive to habitat changes, 6) taxa must have potential economic importance, and 7) sensitivity relations to other related and unrelated taxa should be known.

Considering condition 6, using soil invertebrates with a carnivorous feeding habit as a study group may be less appropriate, for example when evaluating soil improvement applications in agricultural districts. As most of them can be described as surface dwelling individuals, their direct contribution to soil aeration and the degradation of organic matter is less important.

In the context of the study purpose, we could add some additional stipulations. It seems obvious that the chosen taxonomic group should demonstrate low dispersion abilities. This is particularly important when the reference and treated soil areas are rather small. If this condition is denied, community data at both sites can be biased due to migration activities of non-resident species and individuals (8). Also, a relatively high vulnerability of the taxonomic group towards heavy metals is desirable (9).

In our opinion, the last two conditions are the most important criteria when studying soil restoration. An ideal taxonomic group, which meets all the conditions mentioned will be hard to find; we will therefore only discuss some taxa concerning their application abilities. Several field studies allow a comparison of the vulnerability of soil invertebrates.[21,28,31,33] Oribatid mites and earthworms are rather vulnerable whereas springtails and spiders are less suitable according to this criterion. The taxonomy of oribatid mites is not well established as many species Have yet to be described. The high vulnerability, intrinsic diversity and the variation in specialization within species belonging to this taxon does, however, offer good opportunities for the future.[9,28,33,59,96,97] Woodlice occupy an intermediate place regarding their sensitivity,[21] but their low intrinsic diversity and high motility make them less suitable for monitoring community effects.



7.5.1.2. Metal Concentration Measurements in Soil Invertebrates as a Monitoring Tool

*A. Internal Metal Concentrations As Such*

The determination of internal concentrations in soil invertebrates at several time intervals is a useful method to estimate the decrease of internal concentrations after a soil treatment. Comparison of soil invertebrate concentrations between an untreated reference area and a treated area at the same time interval is probably even more relevant. When only the treated area is monitored at certain time intervals during the year, a reduced internal concentration due to soil improvement techniques could be obscured by seasonal variations of metal body burdens.[98]

Accurate analytical techniques and specific sample preparation allow for a relatively quick way to determine metal concentrations of soil arthropods in only a few or even one individual.[99] Of course, (adapted) soil invertebrates should be selected that can survive at heavily contaminated areas and by preference in large numbers. In the case of in situ inactivation treatment, a possible improvement can already be detected; at the moment vegetation recovery is still lacking.

As even closely related species can exhibit different accumulation patterns,[55,100] it is strongly recommended to concentrate on one species. Whether a deconcentrator or a macro- or microconcentrator of heavy metals should be used as the monitoring instrument appears to be an important point of discussion. Use of the heavy metal-accumulating woodlice has been suggested against, as internal concentrations appear to be size- and age-dependent and no equilibrium will be reached.[101] Statistical variance around the mean internal metal concentration for populations of a heavy metal concentrator, could indeed turn out to be relatively high. As a result, statistical differences between populations, originating from contaminated areas with different treatments, will be difficult to prove. Using springtails probably is the best solution as it has been demonstrated that, in spite of their regulatory abilities, internal metal concentrations differ significantly between differently exposed populations.[42] A species eliminating metals in a very strong way should, however, be avoided. Otherwise, the internal concentration will be independent of the external concentration and gives no information about a possible change of the external availability of the metals in the soil.[39] As the elimination rate differs between metal species within a given species, both the biological and the chemical species should be selected during the set up of a monitoring program.

*B. Energy Reserves and Internal Metal Concentrations*

Even if soil improvement activities result in a decrease of internal metal concentrations in soil biota, such as soil invertebrates, the contribution towards the well being of field individuals is more difficult to assess. The relevance of internal metal concentrations can be strongly increased when combined with a simultaneous analysis of energy reserves. The amount of proteins, lipids and glycogen can be regarded as an ecologically relevant measure for the energy budget available for survival, reproduction, growth and storage capacity of energy reserves itself. This method has been successfully applied for the isopod *Porcellio scaber* collected from field populations with a different exposure level towards heavy metals.[37] Decreased energy reserves due to increased heavy metal concentrations have been observed for the population with the highest internal metal concentration. Within this

population, the largest individuals with the highest metal concentrations were most affected. This method is particularly interesting because it provides an opportunity to reveal whether the soil invertebrate community is still affected. We suggest analyzing individuals of a heavy metal-concentrating representative of the soil invertebrate community for their internal metal concentration and energy reserves. When energy reserves decrease with increasing size, negative effects will still take place at the treated area (Fig. 7.3.A). Theoretically, the analysis of only one population at one place could reveal sufficient information. A similar examination of a population of a deconcentrator species will be inadequate, as shown in Figure 7.3.B. At the contaminated reference site, it is possible that the internal metal concentration level affects the energy level, but this can only be proved in comparison with a treated reference site.

A limitation of this approach is a possible size-dependence of the energy content of an individual per unit of weight as a result of factors not related to internal heavy metal concentrations.

*C. Critical Body Concentrations*

It is generally accepted that the internal concentration of a toxicant in an organism determines the ultimate effect.[102] Some models have been proposed to estimate the internal metal concentration that causes mortality from toxicity tests.[103,104] These so-called lethal body concentrations (LBC) have been calculated for only few soil invertebrate species.[59] Internal threshold concentrations (ITC) can be defined, not only for lethality, but also for sublethal effects. The advantage of the use of ITC values is that they are supposed to be invariant to the experimental conditions set up to calculate them. Van Straalen[39] suggests comparing the internal concentration of field individuals with their internal threshold concentrations for sublethal effects. He defines the risk ratio as a quotient of the observed residue (Q) in a species, and the internal threshold concentration (ITC) for that species (Fig. 7.4). The closer the risk ratio approximates to 1 the higher the population can be considered at risk. This approach has been applied for field populations of soil invertebrate species regulating and accumulating heavy metals. It was concluded that risks at heavy metal-contaminated areas were significantly higher compared with a reference site. Risk ratio differences between the species tested were relatively low, suggesting that both metal regulating or metal accumulating species can be used.

### 7.5.2. Toxicity Testing

In this section some considerations on the use of bioassays with soil invertebrates within the scope of soil remediation of heavy metal-contaminated soils are given. As in the assessment of adverse effects of chemicals in general, the species selected for evaluation studies should be representative for the soil invertebrate community in the ecosystem that is subjected to remediation. The measured responses should be ecologically relevant. The species used in the tests can vary depending on the ecosystem studied and its anthropogenic or ecological functions. Including species of different taxonomical and ecological groups and different trophic levels will strengthen the test results (section 7.1). The practicality of culturing (and feeding) and potentials for standardizing the test and test organisms restrict the choice.

A few reviews of tests with soil invertebrates for the assessment of adverse effects of potential toxicants are available.[105-107]



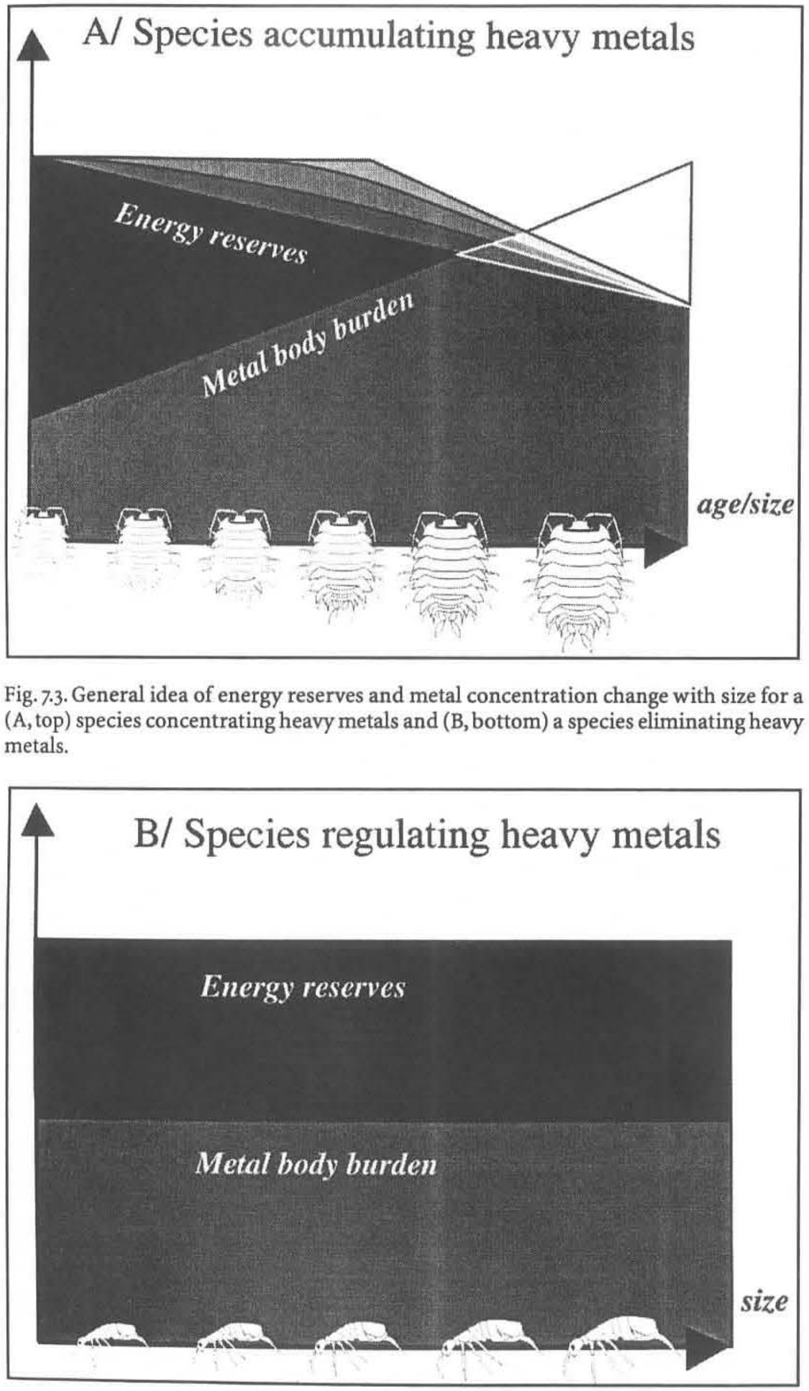


Fig. 7.3. General idea of energy reserves and metal concentration change with size for a (A, top) species concentrating heavy metals and (B, bottom) a species eliminating heavy metals.

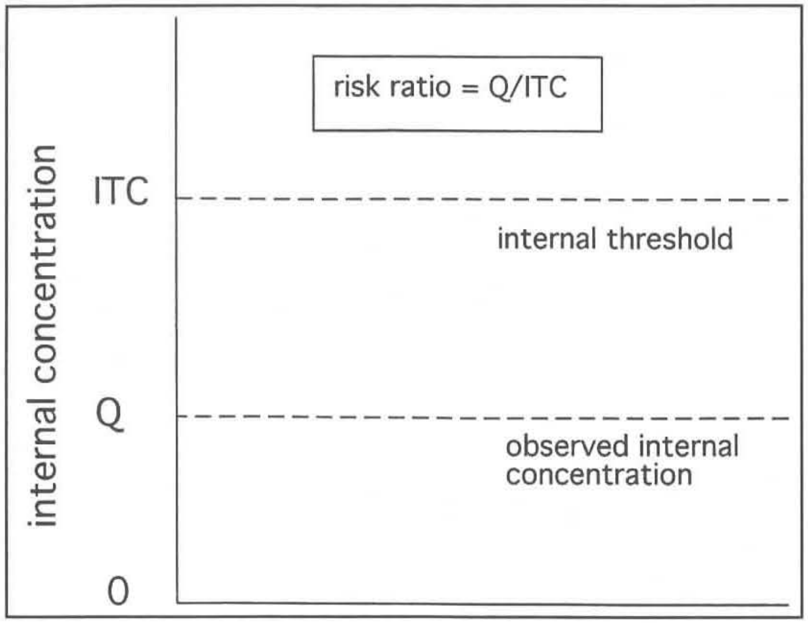


Fig. 7.4. Schematic presentation of the risk ratio for a soil invertebrate population. The shorter the relative distance of the observed residue (Q) to the internal threshold (ITC) for the population, the higher the risk.[40] Reprinted from Bioindicator Systems for Soil Pollution, 1996:5-16, van Straalen NM, Krivolutsky DA, eds, graph 3, with kind permission from Kluwer Academic Publishers, Dordrecht, The Netherlands.

*7.5.2.1. Meso- and Macrofauna Tests*

At present only the test using earthworms (*Eisenia fetida/andrei*) with endpoints survival and biomass is internationally standardized.[108,109] Another test using the springtail *Folsomia candida* with endpoints survival and reproduction is subject to international standardization.[110] A refinement of the earthworm toxicity test with test endpoints survival, growth and reproduction is laid down in a draft international standard.[111] The standardized test systems with earthworms and springtails use an artificial soil with standardized soil characteristics as pH, clay and organic matter content.[108-110] A direct implementation of these tests in the evaluation of decontaminated soils is not possible. The tests can be adapted for risk assessment of contaminated, decontaminated or remediated soils. The fact that results from the classical tests can be extrapolated to natural soils using sorption data, is an advantage to bioassays that use different organisms.[112] However, the impact of aging and different chemical forms of metals in the field may hamper a direct comparison of laboratory and field soils.[52]

A variety of tests focusing on other impact levels with different endpoints are under development. The possible use of stress proteins (metallothioneins, Hsp70 and Hsp60) in soil invertebrates as biomarkers for environmental stress has gained



much interest in recent years.[67,113,114] Also, lysosomal integrity has been proposed as a biomarker for toxicity in soils.[115,116] Ultrastructural alterations have been proposed as biomarkers in soil invertebrates as well.[90,117]

*7.5.2.2. Experimental Set Up in Soil Remediation Studies*

In the assessment of the toxicity of metal residues in remediated soils, toxicity tests and bioassays in the laboratory should be combined with in situ experiments. Laboratory tests, mostly on single species, have the advantage that they can be carried out under controlled environmental conditions. The use of species used in classical toxicity testing of toxicants can be valuable because more data are available for comparison.

Special attention should be paid to differences between artificially contaminated soils or soils collected from the field. Significant differences of metal availability and toxic effects between artificially contaminated soils and contaminated field soils have been found.[52,118,119] Tests which are performed with artificially contaminated soils generally will overestimate the availability and toxicity. These results can be regarded as 'the worst case.' Artificially contaminated soils are useful in the evaluation of soil additives for instance. Evaluation of the effects of in situ soil remediation is preferably carried out with the field soils considered. Non-homogeneous distribution of the heavy metals in the field can be a problem and force investigators to mix soils or use a high number of direct soil samples as replicates.

Sampling procedures of soils should be standardized and performed in such a way that disturbance of the soil is minimal. Different sampling techniques, such as core sampling versus shovelling, can strongly influence the external available metal fraction and metal speciation. The storage of the soil or pretreatment will affect the oxygenation of soils and can cause changes in bioavailability of metals in the samples and, as a consequence, affect toxicity.

Because many toxicity tests with soil invertebrates are still under development, a first step of major importance in a sequential evaluation of soil remediation studies is the standardization of toxicity tests in the laboratory. These tests must lead to the determination of short and long term effects on soil invertebrates. The use of artificially contaminated soils under standardized conditions facilitates the determination of correlations between effects and external and internal concentrations. The use of external or internal concentrations as a monitor for bioavailability can be evaluated. In a second phase, bioassays with a gradient of contaminated field soils should be carried out with the test species in the laboratory. The toxicity of metal mixtures in the field can be measured in these bioassays. Because of synergetic and antagonistic effects, the toxicity of the field soils cannot be predicted from toxicity tests with specific metals. Indications of antagonistic effects have been mentioned between Zn and Cd for springtails[120] and for a mixture of Co, Zn and Cd for an earthworm species.[121,122] Another important factor to be evaluated in bioassays is the aging of the field soils, which results in differences in bioavailability. In this stage the formerly established monitors for bioavailability can be evaluated. The influence of physico-chemical soil characteristics on the availability of metals can be studied as well. Disturbance of field soils by soil sampling and preparation is still a problem, however.

Subsequently, in situ bioassays with 'laboratory' test organisms will elucidate the effects of natural climatological conditions, changing temperature, humidity and photoperiod on the former test results. These kind of experiments are a first step to validation of results from laboratory tests. Aspects such as weathering, leaching, time, and sustainability can be evaluated using in situ bioassays. The influence of a patchy distribution of the metals can be assessed as well. In a final step, local species could be used in bioassays in the laboratory or the field. In this way differences in sensitivity of the local populations and 'laboratory' test organisms can be evalutated.

Differences in response between local populations and populations from uncontaminated or less contaminated sites can be expected. Due to a diminished variance to toxic stress in adapted populations, the maintenance of these populations is uncertain. For example, they might not be able to readapt to lower levels of heavy metals.[22] Differences in biomarker response could be used to differentiate between populations that were already exposed to heavy metal contamination and recolonizing populations from uncontaminated areas.

A sustainable reduction of the bioavailable metal concentrations must form the major goal in soil remediation programs. Monitoring of soil remediation in time will be necessary to evaluate the sustainable character of the techniques being used and the evolution of populations at these sites. Therefore, scientists should pay attention to changes in bioavailability, metal speciation and ecological relevance in soil remediation studies.

## 7.6. Conclusions

Although little is known about soil invertebrate-mediated element fluxes within the soil, it is generally agreed that meso- and macro-invertebrates play an important role in soil functioning. Soil invertebrate taxa positively affect soil fertility due to their motility and variety of feeding strategies, inhabit a broad vertical distribution range and affect functioning of other soil biota by their activity (7.1). When soil restoration techniques are developed and applied, the soil invertebrate fauna itself can and should be used as an evaluation tool. A more or less adopted description of vulnerability of soil invertebrates, as was proposed by van Straalen,[96] can be useful to comprehend the consequences of exposure to heavy metals and the importance of soil restoration treatments. Several mechanisms to cope with an excess of heavy metals exist, ranging from life-history strategy 'correction' as a result of a selection process to the storage-detoxification in macroconcentrators such as *Isopoda* and *Aranea*, or the deconcentration of metals due to midgut epithelium renewal during moulting in Collembola (section 7.4). Body concentrations do not only depend on biochemistry-related physiologyical characteristics as summarized above. The metal fraction within the soil that should be considered for uptake and which is related to ultimate body concentration also depends on other taxon-dependent and soil-dependent characteristics (section 7.3.2). This external availability of metals is expected to decrease when applying in situ inactivation and phytorestoration techniques. Despite the fact that physiological and life-history adaptations (section 7.4) have been demonstrated within soil invertebrates, in situ biological monitoring also showed a reduced density, diversity and energy budget and an enlarged risk ratio (section 7.5.2.2) for many taxa living in metal-contaminated soils. A diminished genetic variability and an increased need for environmental metal availability following strong selection on metal contaminated sites, as demonstrated for *Orchesella cincta* (section 7.4), is a last item that should be dealt with when evaluating factors affecting soil invertebrate vulnerability in a context of in situ restoration. As a result of these adaptation implications, short-term effects following soil 'improvement' treatments could reveal disappointing results, as adapted populations inhabiting heavy metal contaminated areas could demonstrate an increased vulnerability. To start evaluating in situ restoration of soils, using additives or adapted and/or manipulated plants, small experimental fields are suggested. At the moment, bioassays for terrestrial invertebrates remain scarce and the development of protocols for in situ biological monitoring is not considered. However, several opportunities exist to use soil invertebrates as an evaluation tool in the near future (section 7.5).



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